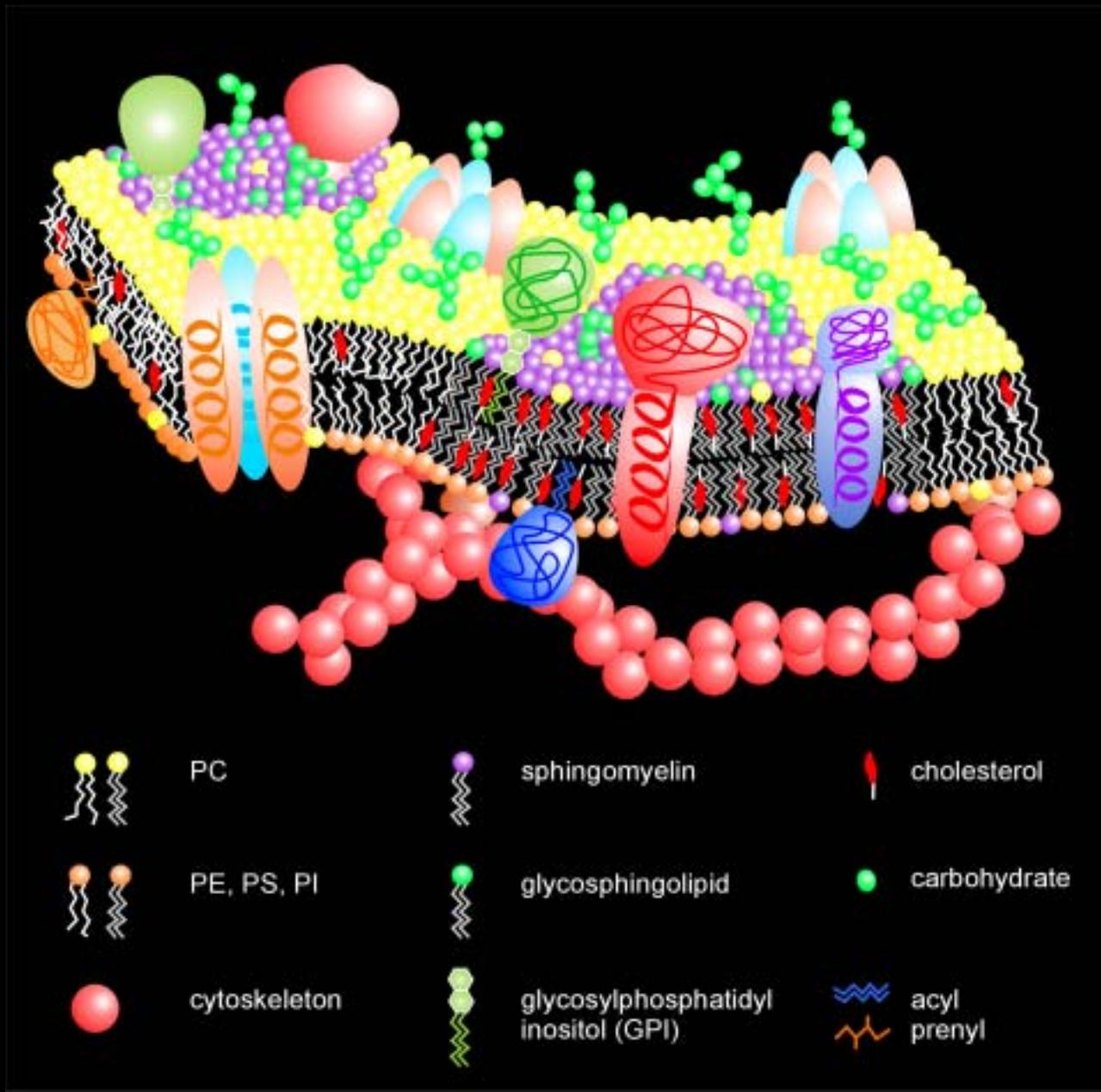


ELASTIC COUPLING OF MEMBRANE PROTEIN STRUCTURE AND STABILITY TO LIPID BILAYER FORCES

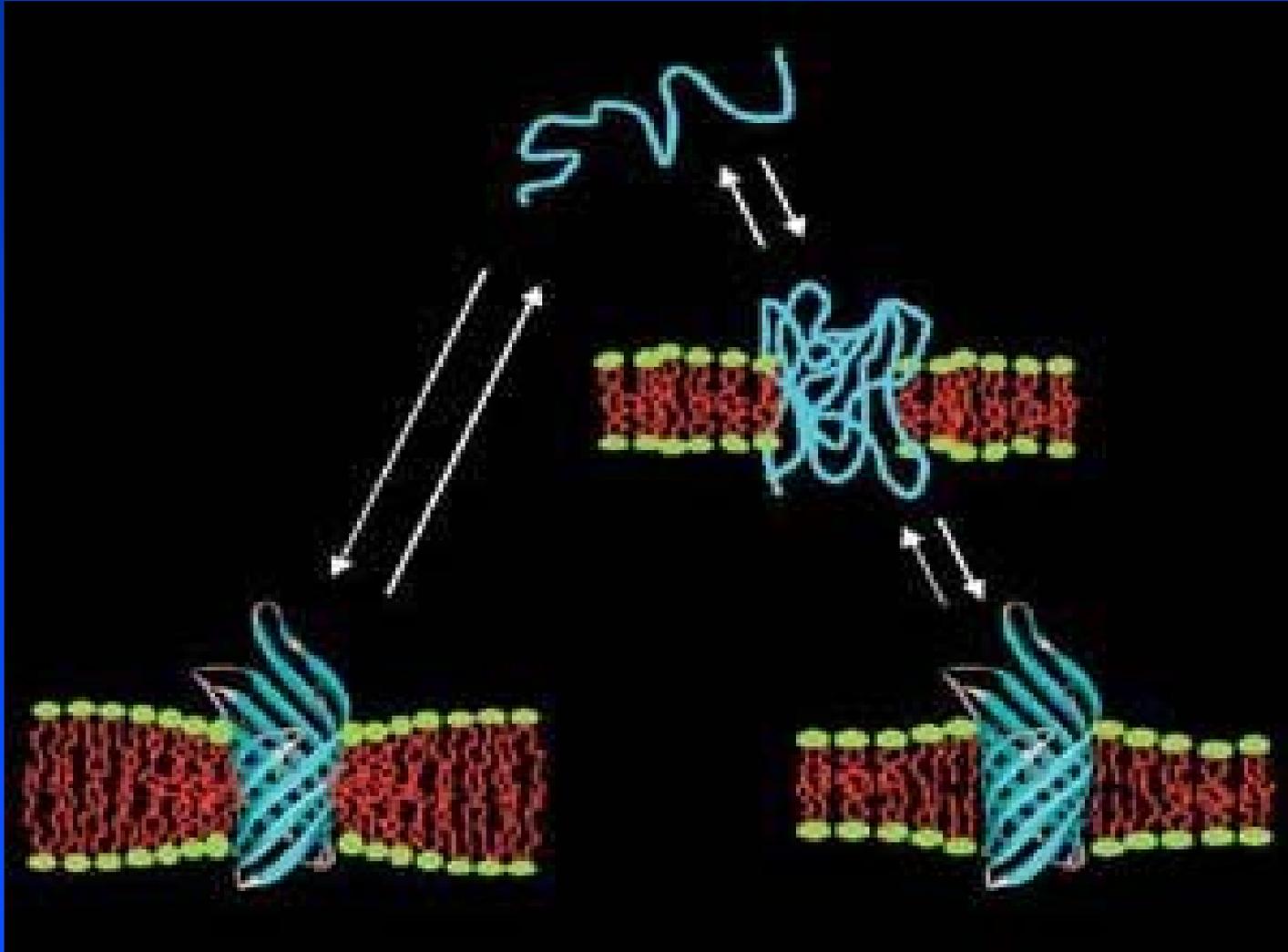


Lukas Tamm
University of Virginia

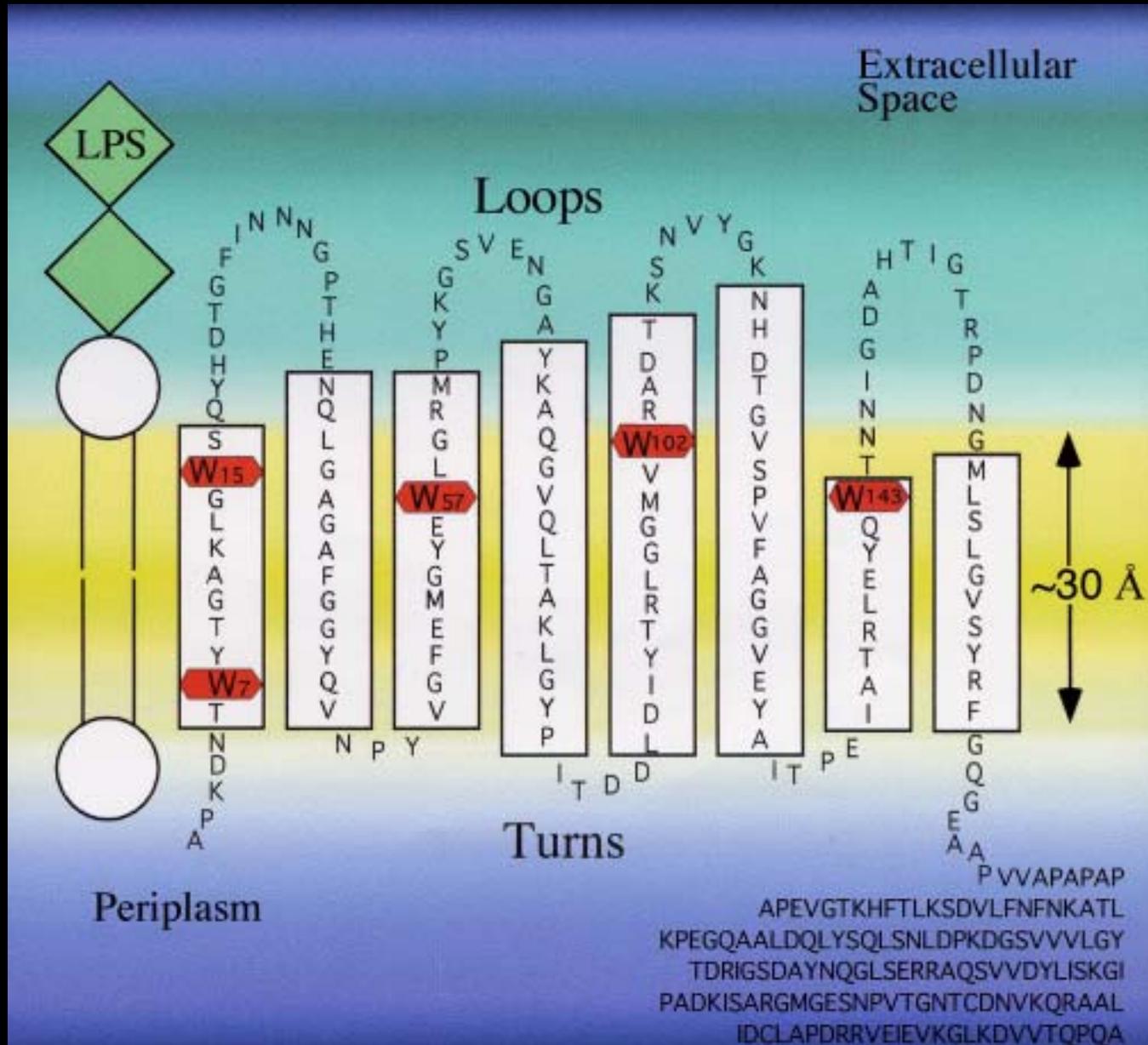
Cell membranes are complex dynamic assemblies



The lipid bilayer modulates thermodynamic stability of OmpA
The structure of OmpA is dynamic
The strength of polar side chain interactions can be measured



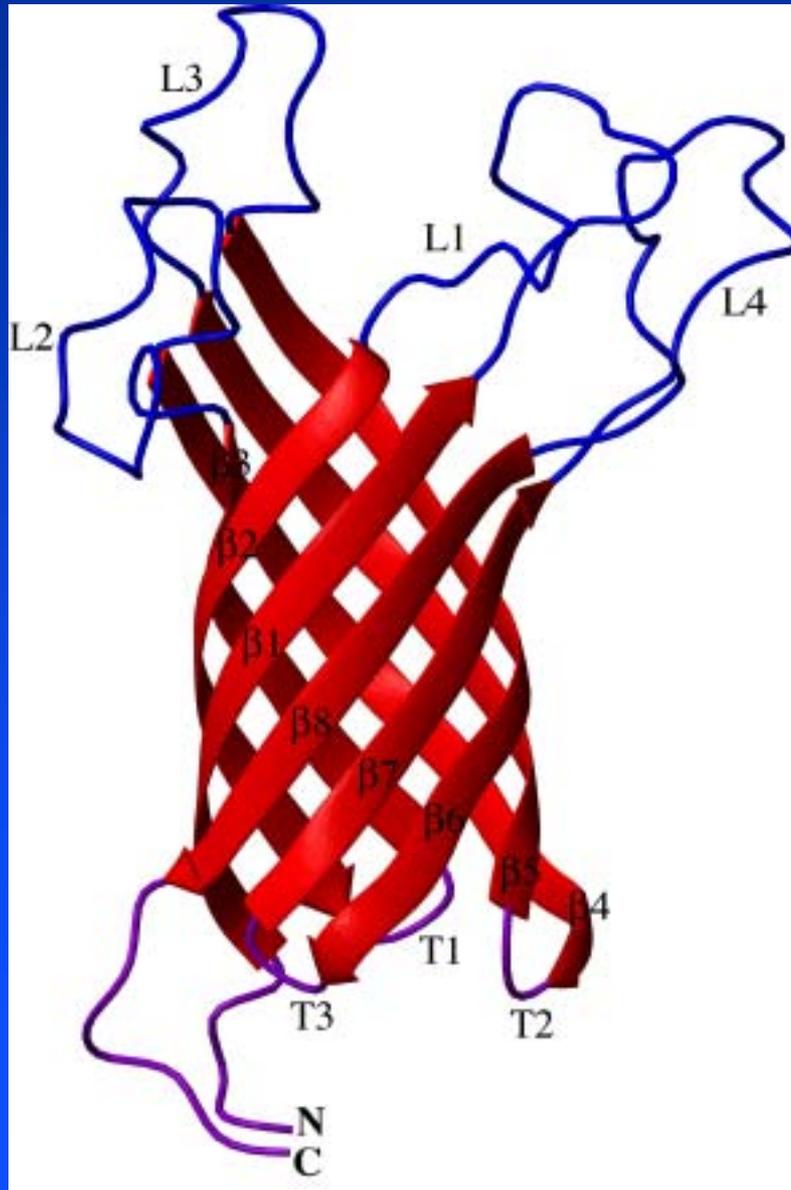
Outer Membrane Protein A (OmpA) of *E. coli*



Functions ascribed to OmpA:

- Structural protein: connects the outer membrane to periplasmic peptidoglycan
- Ion channel: slightly anion-selective
- Receptor for various bacteriophages (K3, O_x2)
- Mediates bacterial conjugation

Fold of the OmpA TM domain by NMR spectroscopy

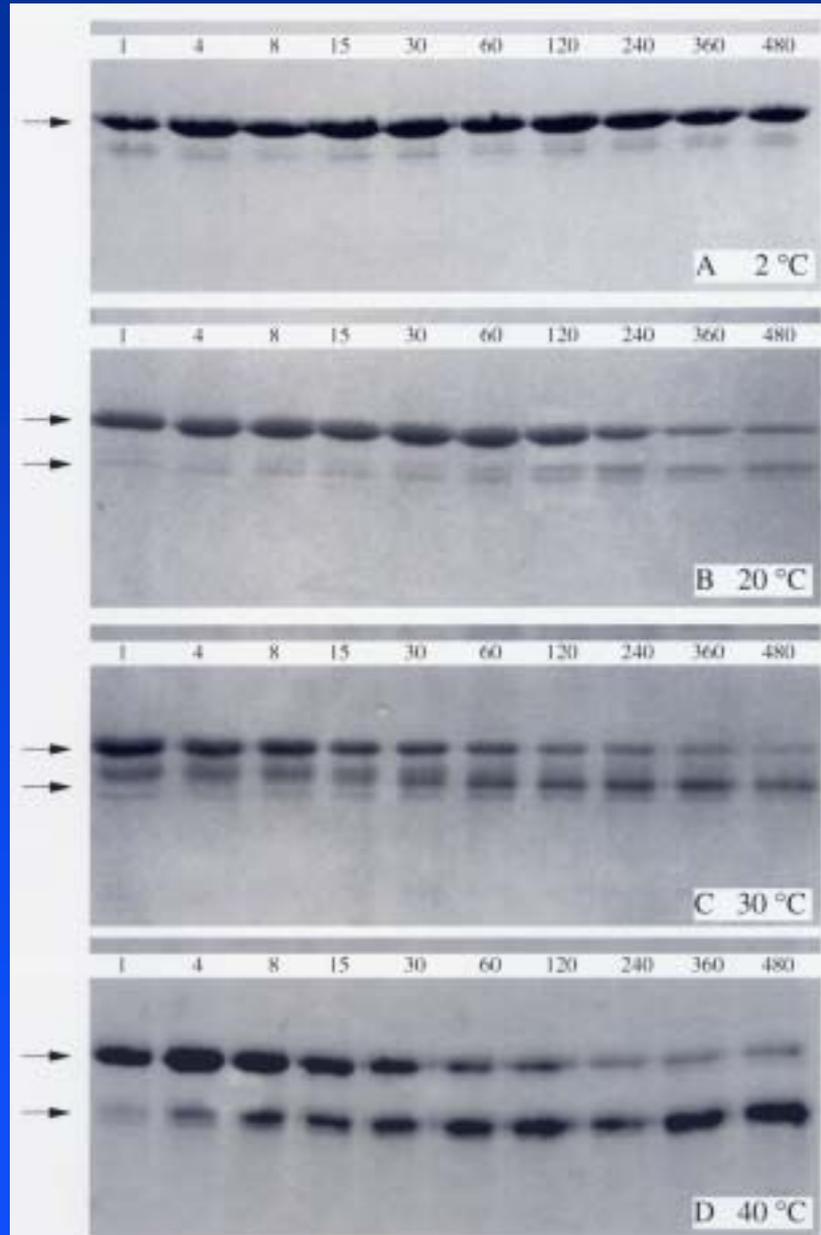


19 kDa
177 residues

in DPC micelle
of ~46 kDa

Expressed and urea-unfolded OmpA can be refolded into preformed DOPC bilayers as monitored here by an SDS gel-shift assay

35 kDa
30 kDa

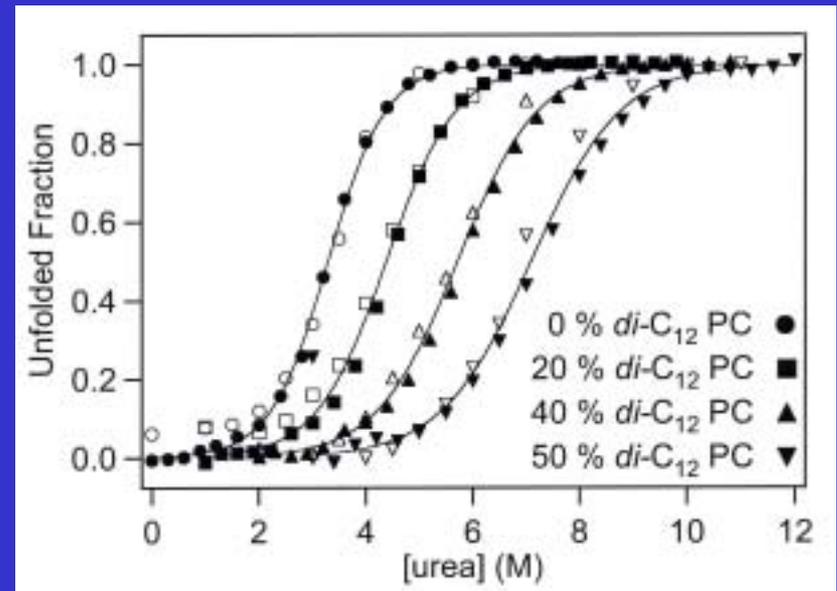
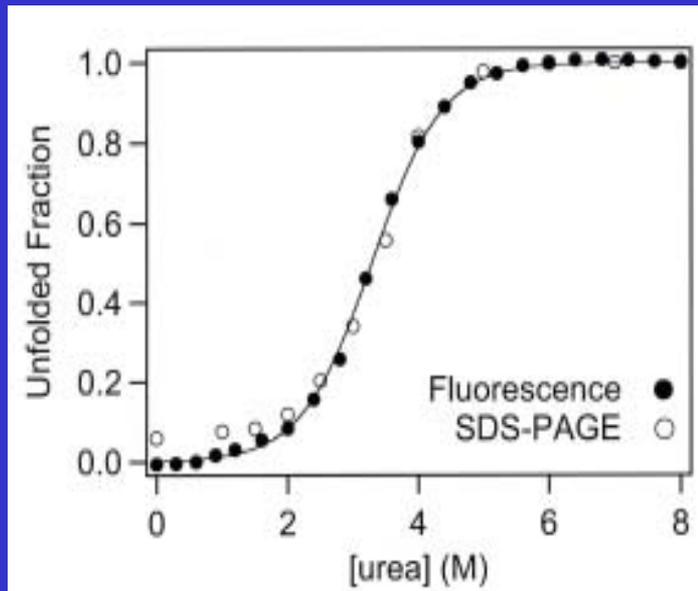
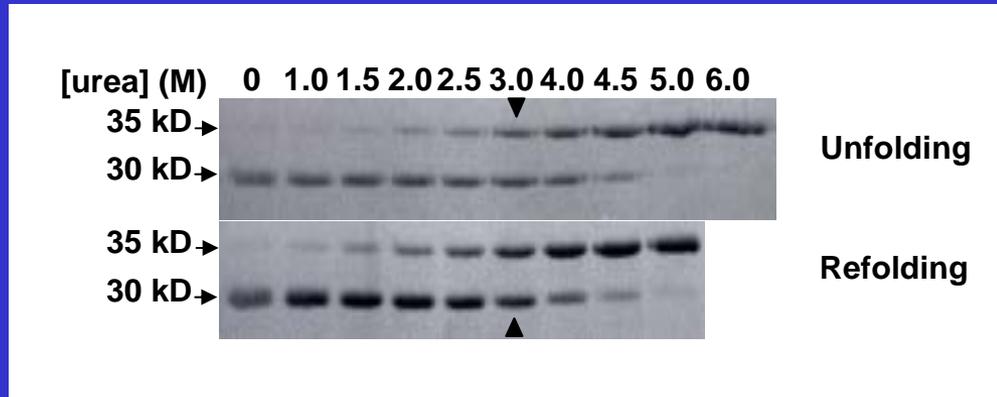


Equilibrium Folding of OmpA

The thermodynamic stability of OmpA is coupled to elastic bilayer forces

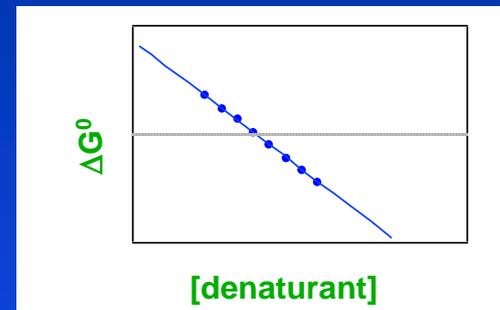
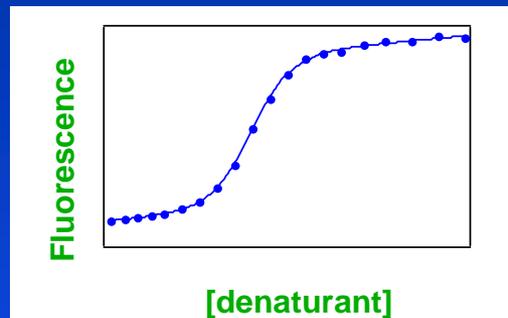
Folding/Unfolding is Reversible and Two-State

same degree of folding/unfolding measured by fluorescence and SDS-PAGE



Measuring the thermodynamic stability of membrane proteins in lipid bilayers: reversible unfolding transition in urea

10 % POPE/
82.5 % POPC/
7.5 % POPG



$\Delta G_{U, H_2O}^0 = 3.48$
kcal/mol
 $m = 1.20$ kcal
 $\text{mol}^{-1} \text{M}^{-1}$

$$F = F_f - (F_f - F_u) \frac{\exp\{m([urea] - [urea]_{50})/RT\}}{1 + \exp\{m([urea] - [urea]_{50})/RT\}}$$

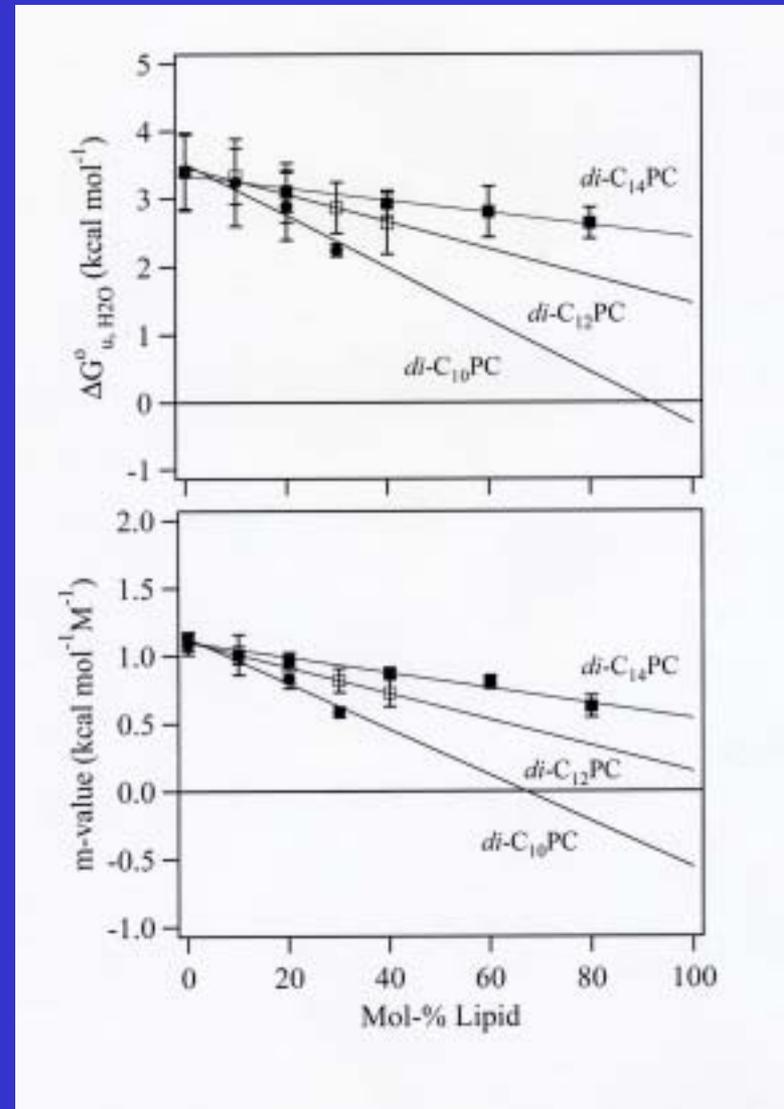
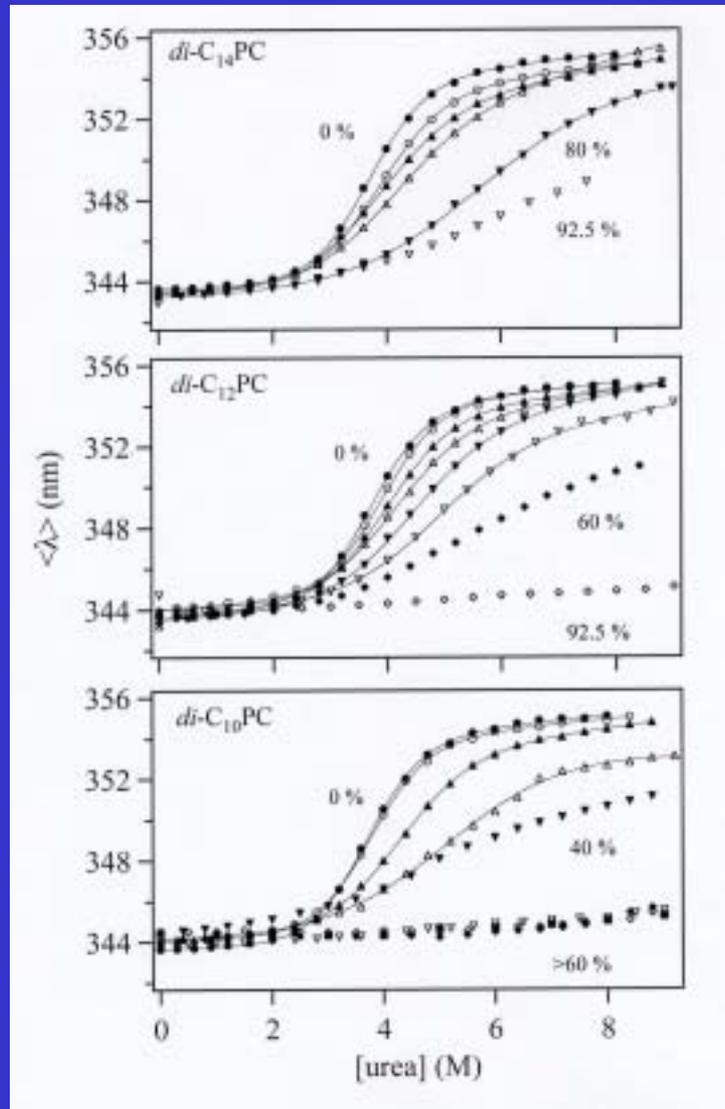
$$\Delta G_u = m[urea]_{50} \quad \text{free energy of unfolding}$$

m is a measure of the cooperativity of unfolding

$[urea]_{50}$ is the urea conc at the mid-point of the transition

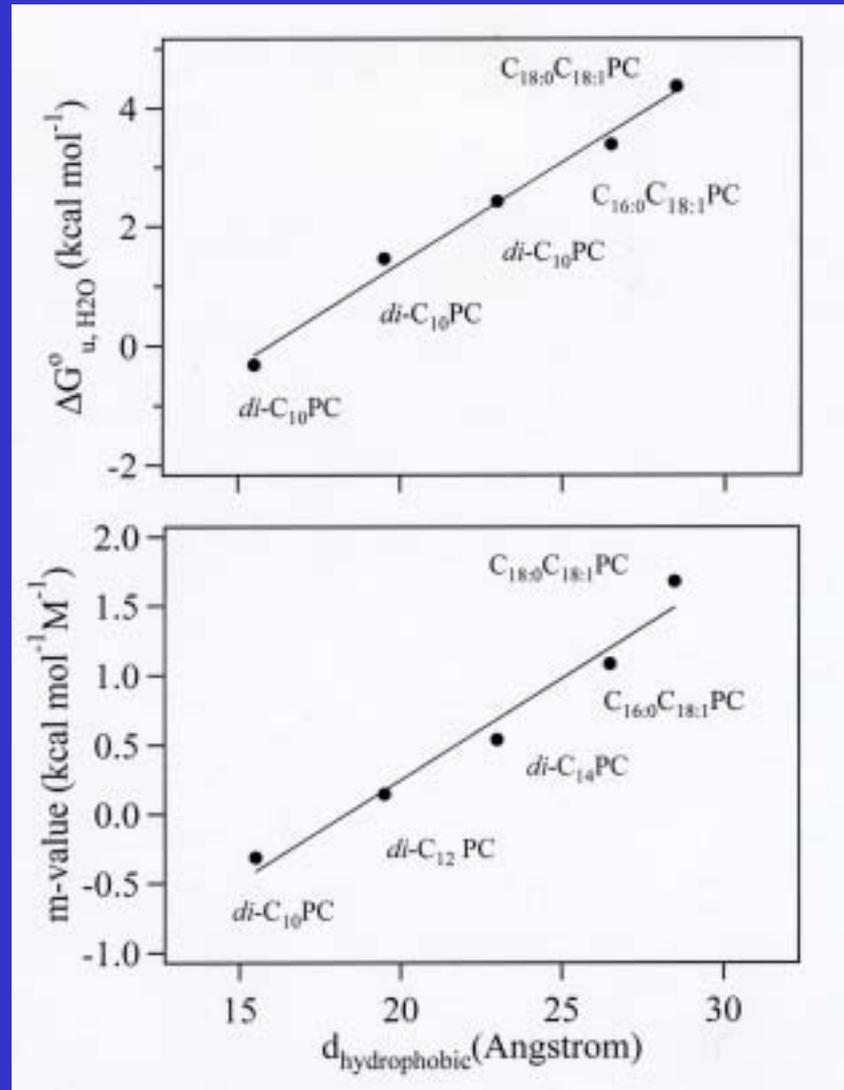
Effect of Bilayer Thickness on Thermodynamic Stability of OmpA

Composition: x % $di-C_n$ PC, (92.5- x) % POPC, 7.5 % POPG; pH = 10.0; Temp = 37.5 °C



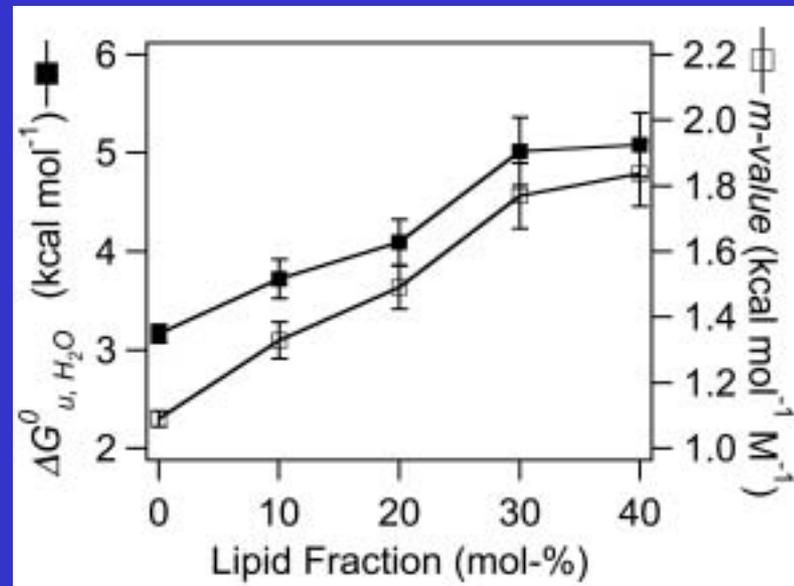
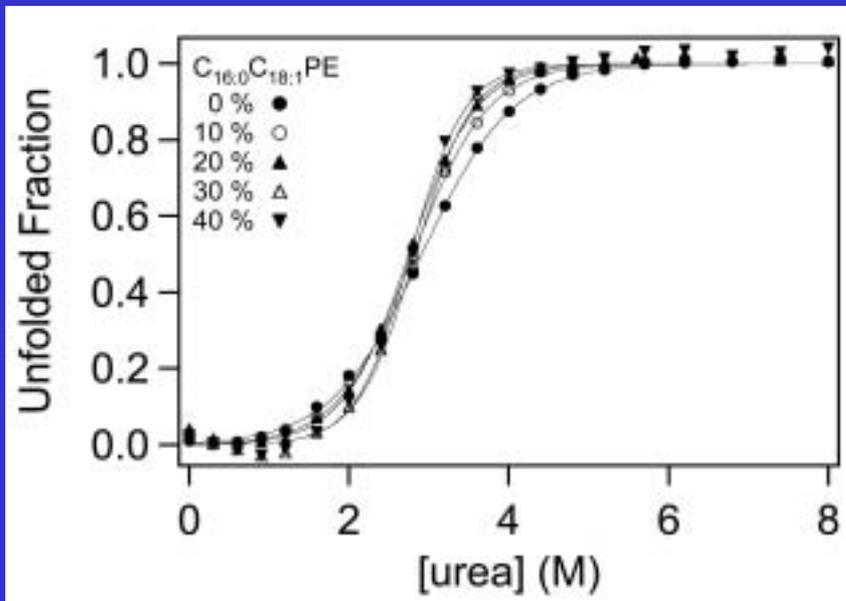
Thermodynamic Stability of OmpA Increases as Bilayer Thickness Increases

Composition: x % $di-C_n$ PC, (92.5- x) % POPC, 7.5 % POPG; pH = 10.0; Temp = 37.5 °C



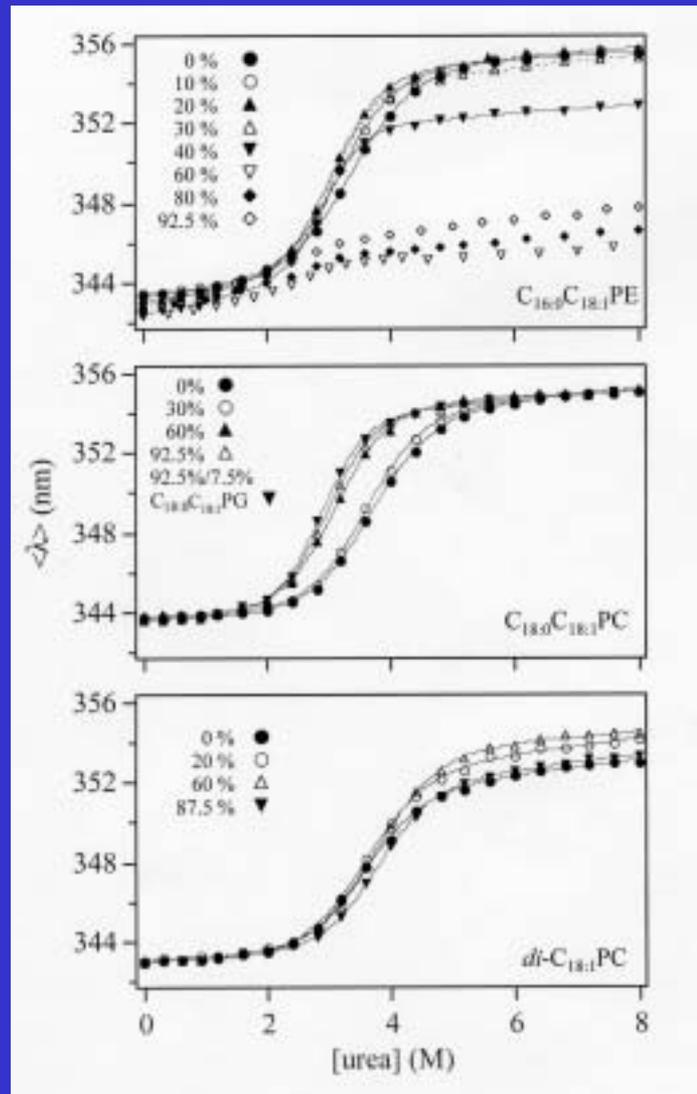
Effect of Phosphatidylethanolamine (POPE) A Cone-Shaped Lipid

Composition: x % POPE, (92.5-x) % POPC, 7.5 % POPG; pH = 10.0; Temp = 37.5 °C



cis-Double Bonds (Internal Membrane Pressure) also Increases Thermodynamic Stability of OmpA

Composition: x % *di*-C_nPC, (92.5- x) % POPC, 7.5 % POPG; pH = 10.0; Temp = 37.5 °C



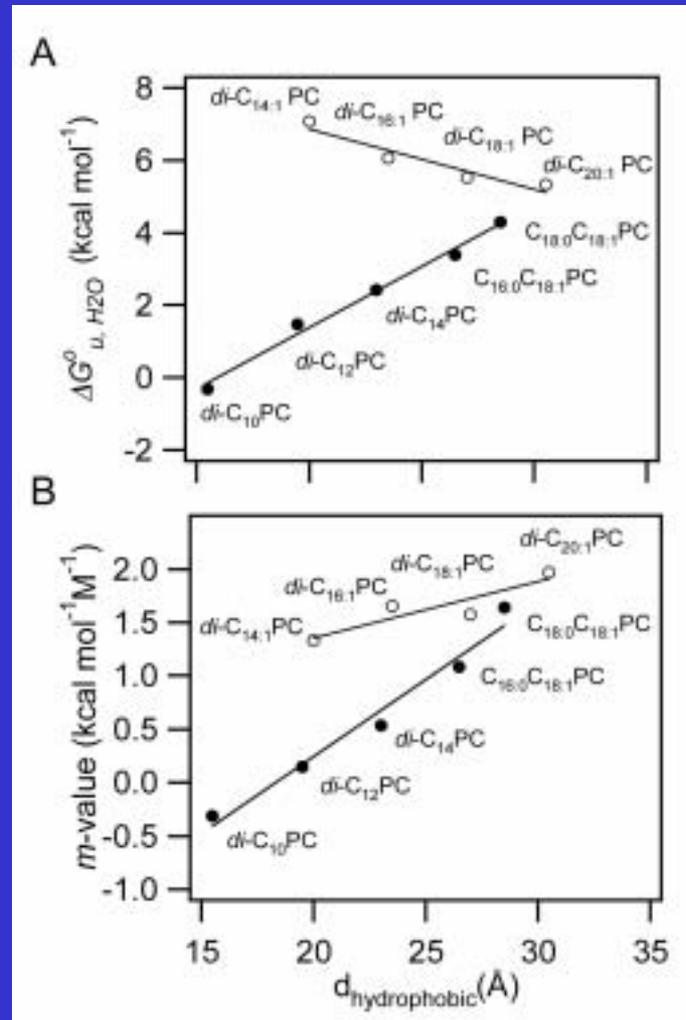
7.5 % POPG

7.5 % POPG

12.5 % POPG

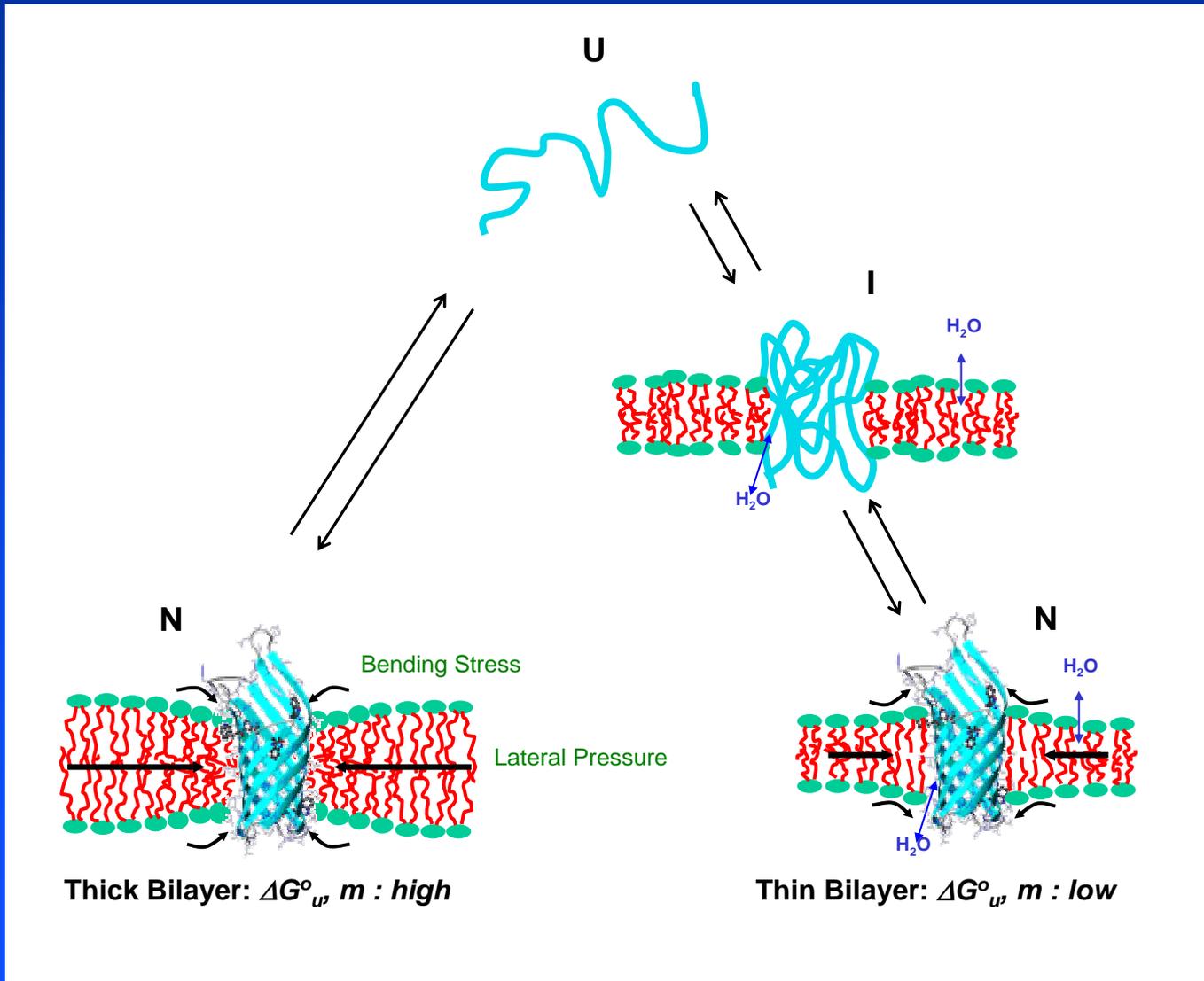
Thermodynamic Stability of OmpA Increases as Lateral Pressure in Bilayer Increases

double unsaturated lipids are increasingly cone-shaped with decreasing bilayer thickness



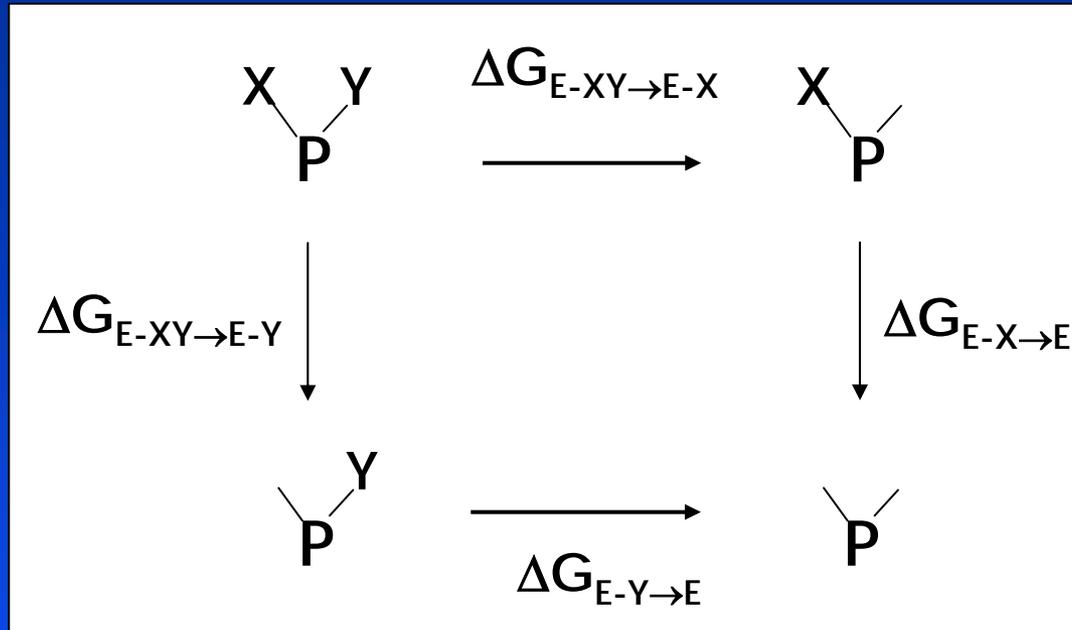
Bilayer Effects on the Stability of OmpA

Thermodynamics of Folding/Unfolding are Elastically Coupled to Internal Bilayer Forces/Pressures



Determining the strength and cooperativity of interacting side chains using thermodynamic cycles

(Horovitz and Fersht *JMB* 1990, 1992)



Definition

in terms of $\Delta G^{\circ}_{\text{unfolding,H2O}}$

$$\begin{aligned} \Delta\Delta G_{\text{coupling X-Y}} &= \Delta G_{E-XY \rightarrow EX} - \Delta G_{E-Y \rightarrow E} \\ &= \Delta G_{E-XY \rightarrow EY} - \Delta G_{E-X \rightarrow E} \end{aligned}$$



$$\begin{aligned} \Delta\Delta G_{\text{coupling X-Y}} &= [\Delta G^{\circ}(XY)_U - \Delta G^{\circ}(X0)_U] - [\Delta G^{\circ}(0Y)_U - \Delta G^{\circ}(00)_U] \\ &= [\Delta G^{\circ}(XY)_U - \Delta G^{\circ}(0Y)_U] - [\Delta G^{\circ}(X0)_U - \Delta G^{\circ}(00)_U] \end{aligned}$$

The energy terms involving the interactions between the mutated sites and their environment cancel!

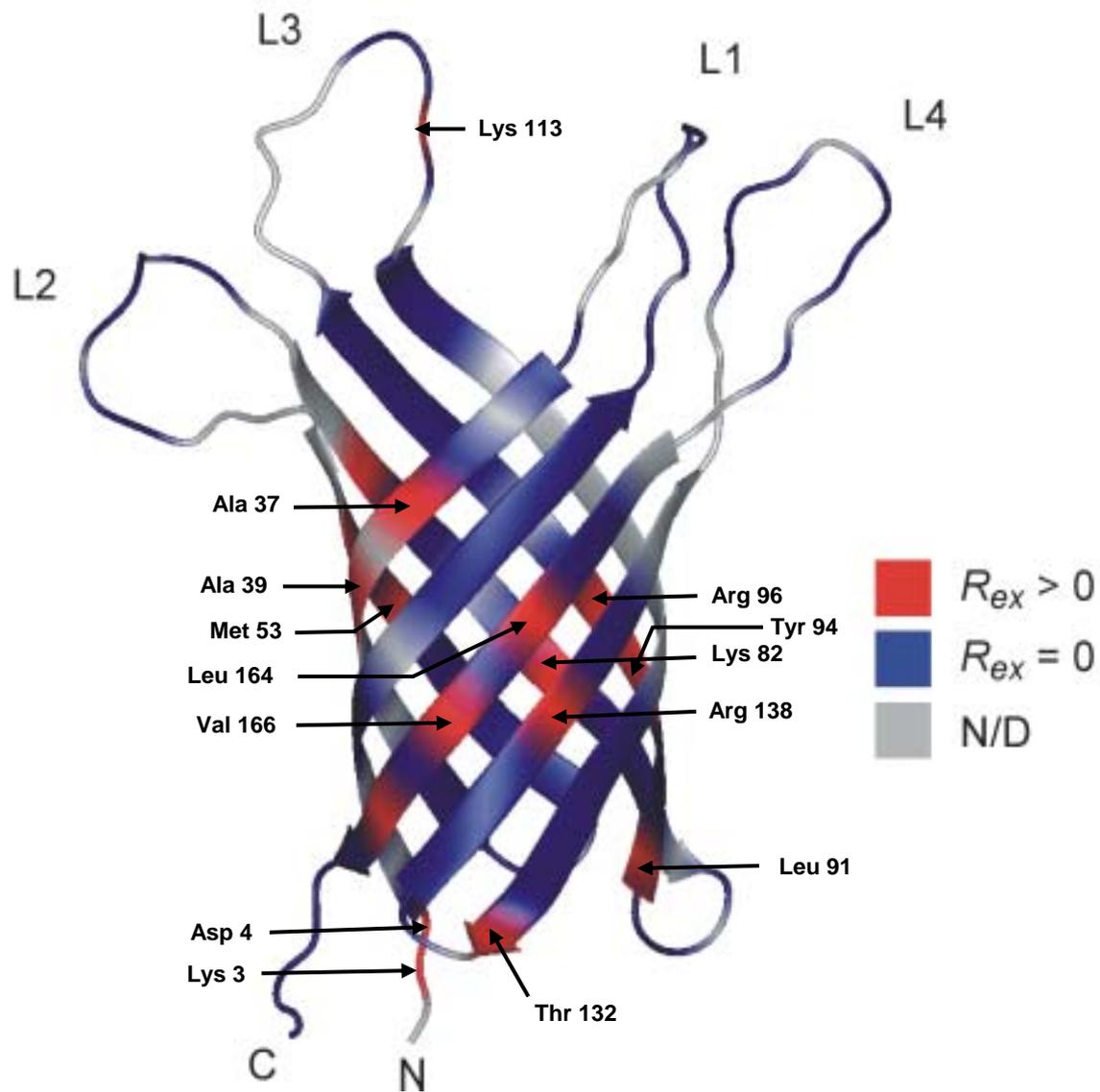
Backbone Dynamics by NMR

what can we learn about function
of membrane proteins from
dynamics measurements?

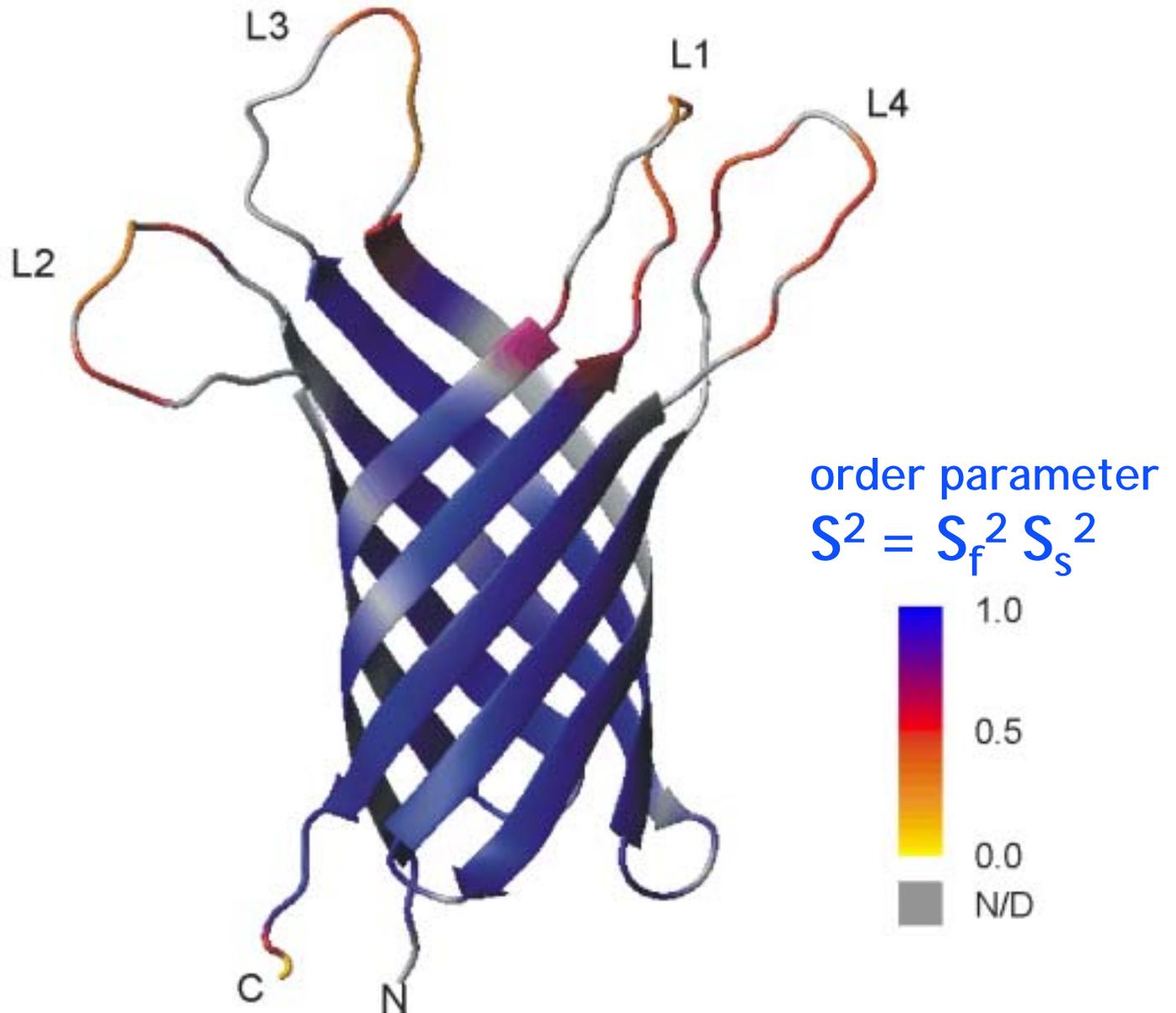
measure ^{15}N T_1 , T_2 , and $\{^1\text{H}\}$ - ^{15}N NOEs at 500,
600, and 750 MHz

analyze data to get motional correlation times,
order parameters, and conformational
exchange factors

Exchange Factors, R_{ex} conformational exchange on μ s-ms time-scale



Dynamic Structure (ps and ns) of OmpA TM Domain



Thanks to:

NMR Structure and Dynamics of OmpA

Ashish Arora

Binyong Liang

Dennis Rinehart



Folding of OmpA

Heedeok Hong

Jörg Kleinschmidt

Natalia Rodionova



Single Channel Measurements of OmpA

Ashish Arora

Collaborators:

John Bushweller, Gabor Szabo (U. of Virginia)

Frits Abildgaard, Heike Blad (NMRFAM, U. of Wisconsin)

Richard Wagner (U. of Osnabrück)

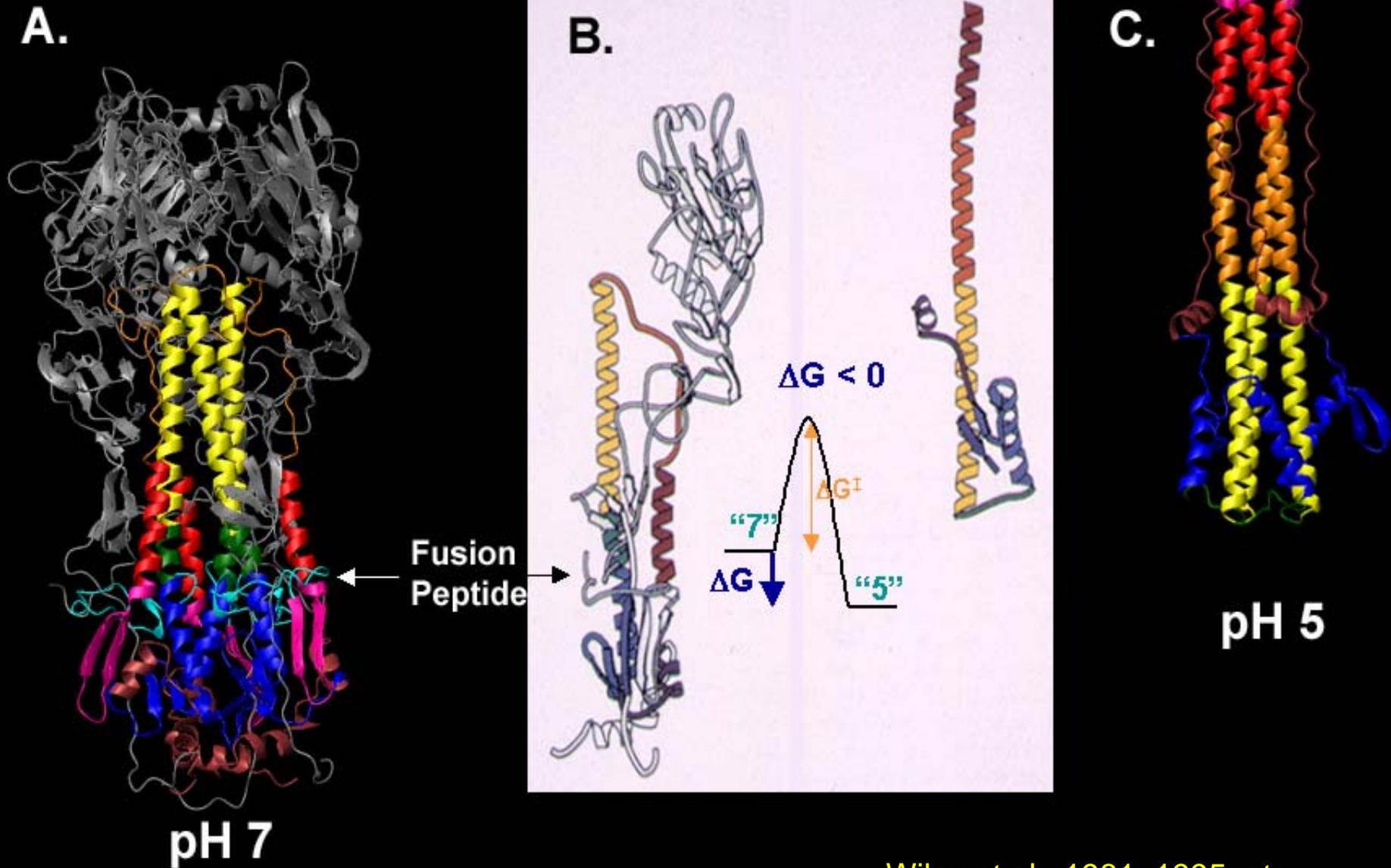
Funding:

NIGMS

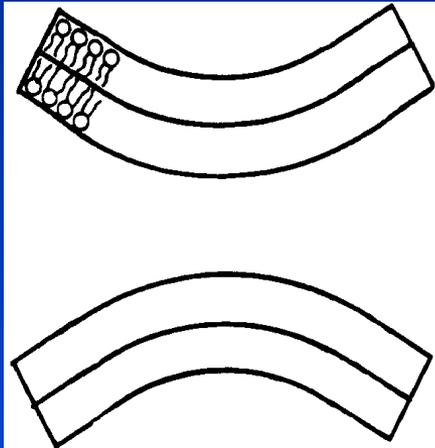
MEMBRANE FUSION: Influenza Virus



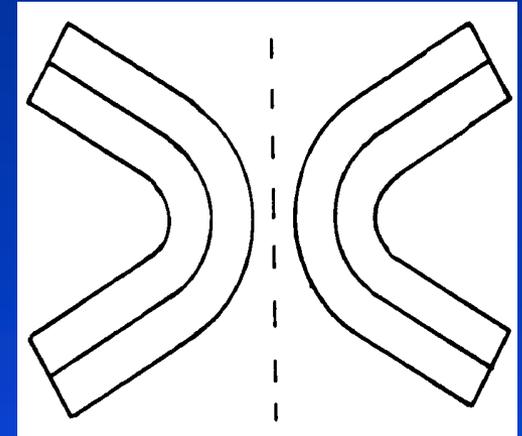
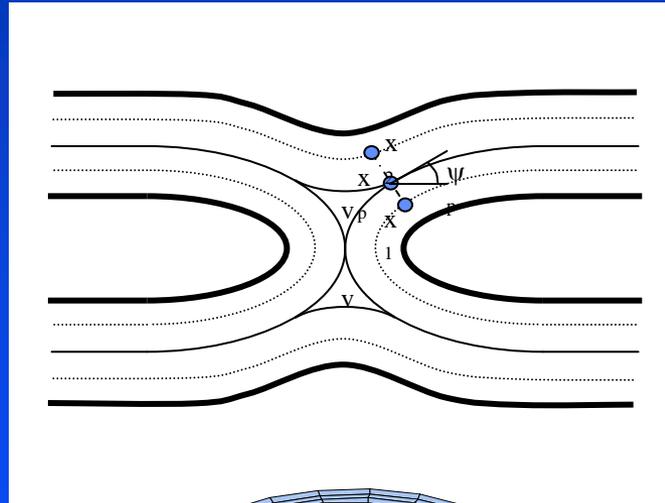
"Spring-loaded" pH-dependent conformational change of influenza HA



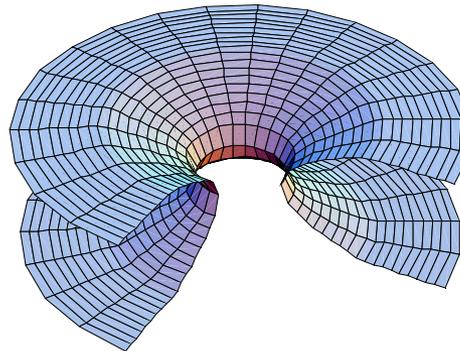
Membrane Fusion



two approaching membranes



fusion pore

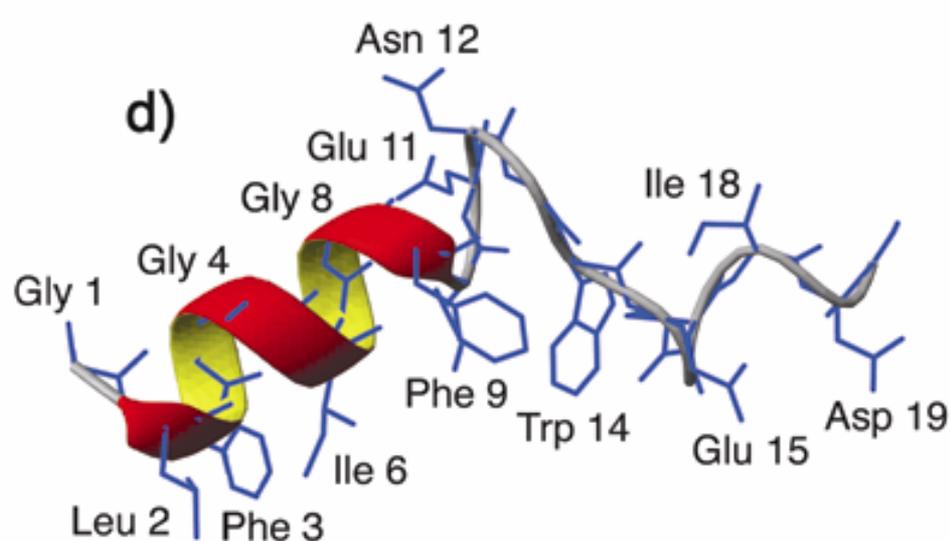
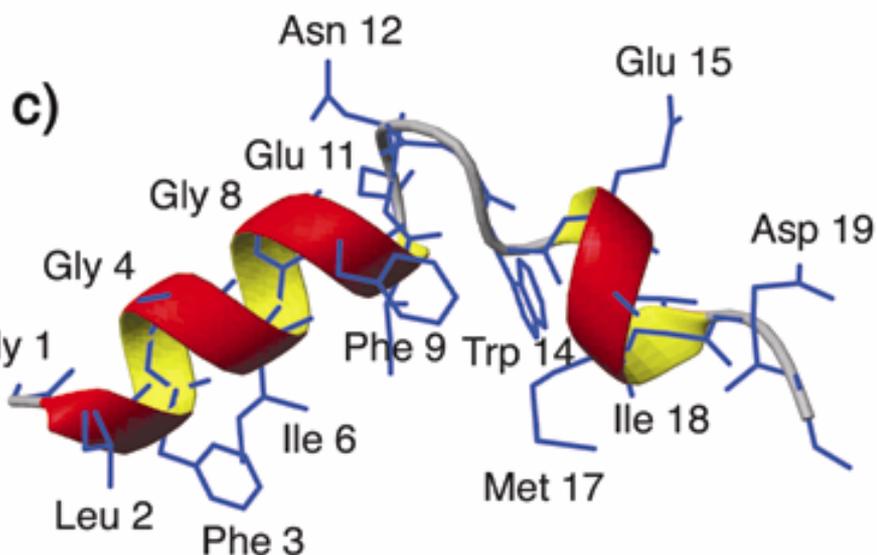
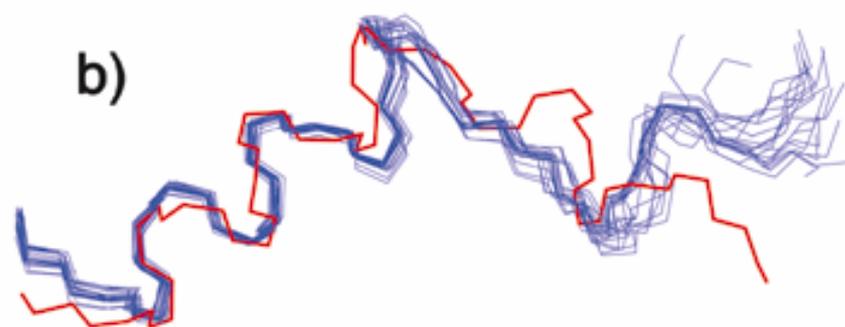
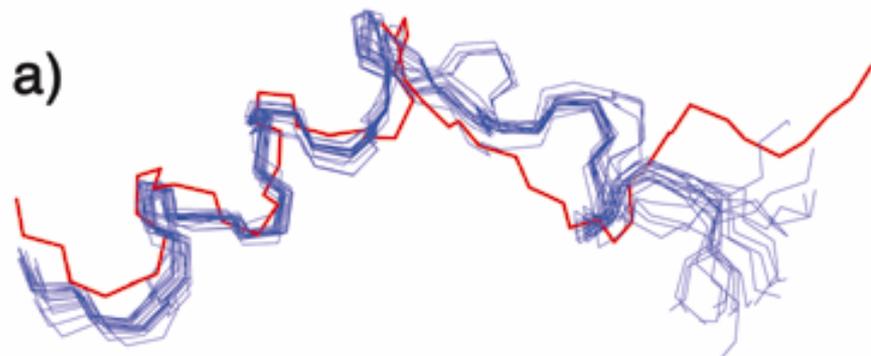


“stress-free” stalk intermediate
Albanesi & Markin, 2002

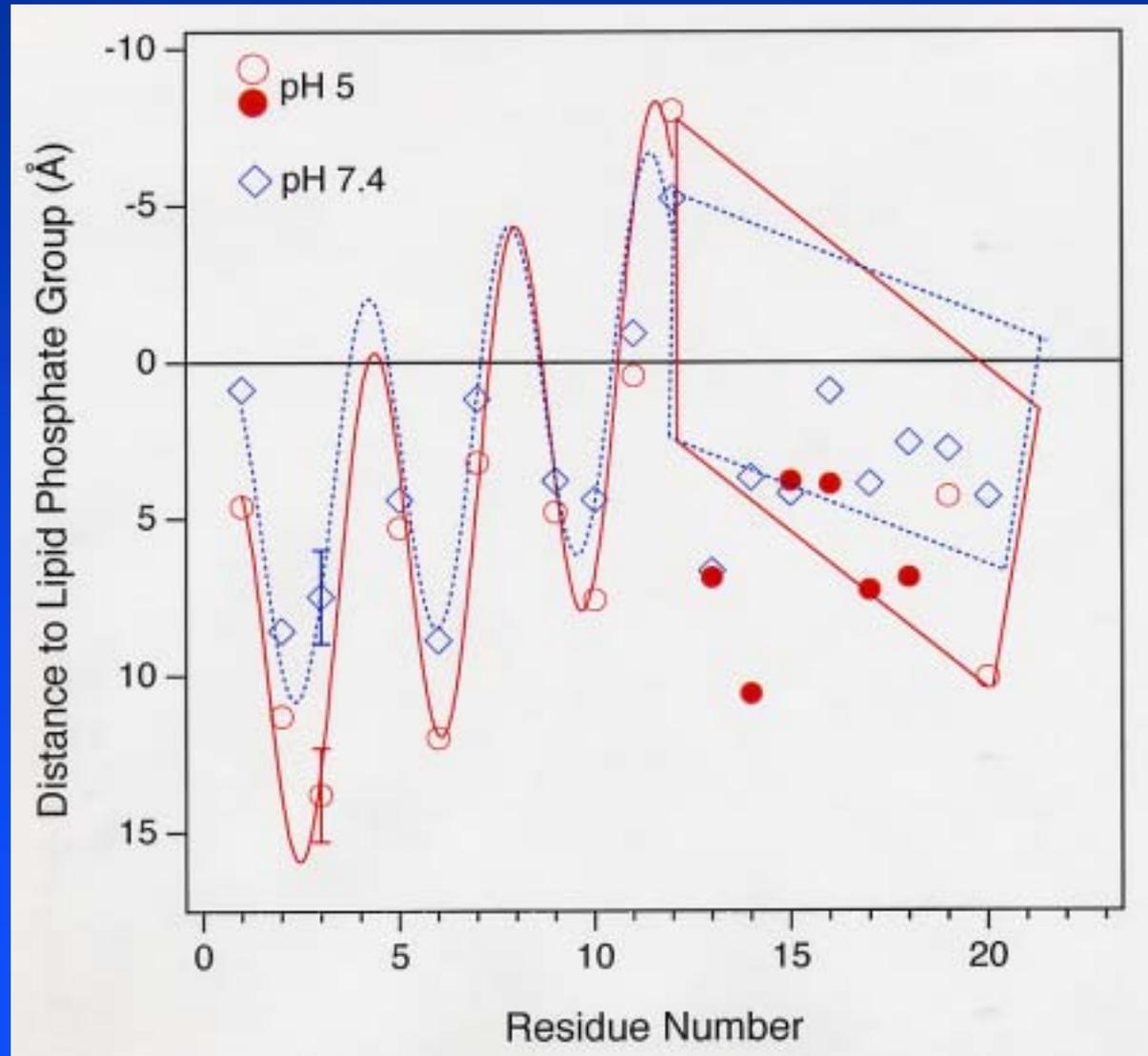
NMR Structures of Influenza Hemagglutinin Fusion Peptide in DPC Micelles

pH 5

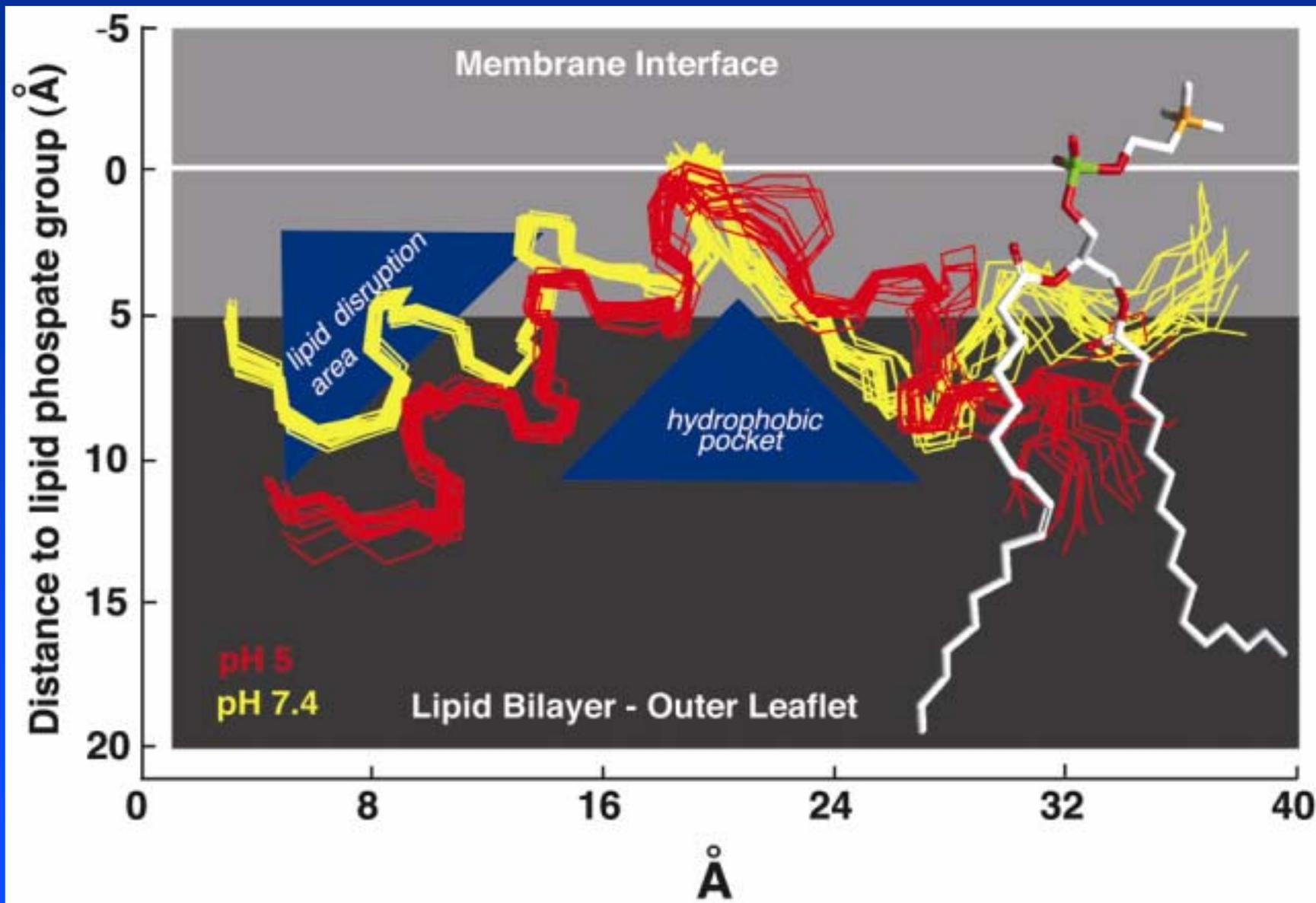
pH 7.4



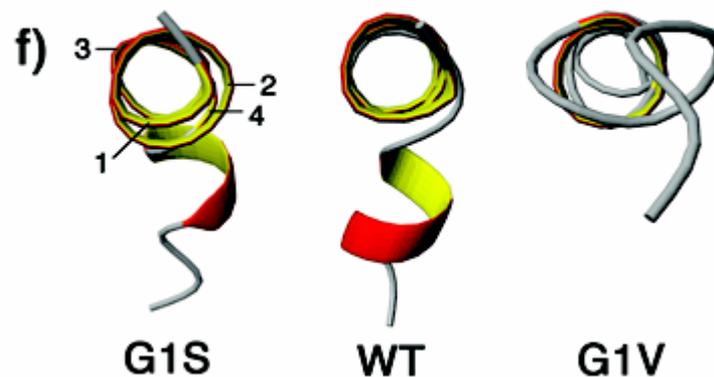
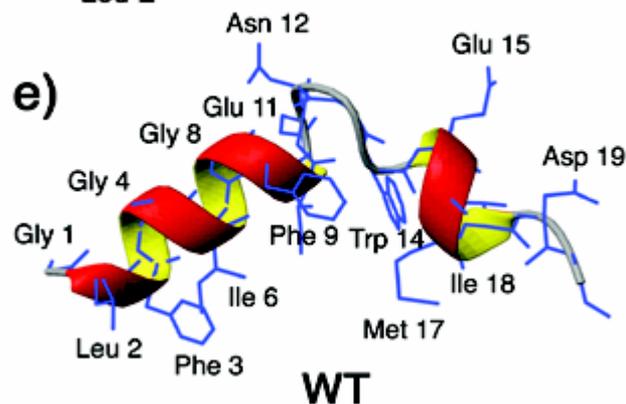
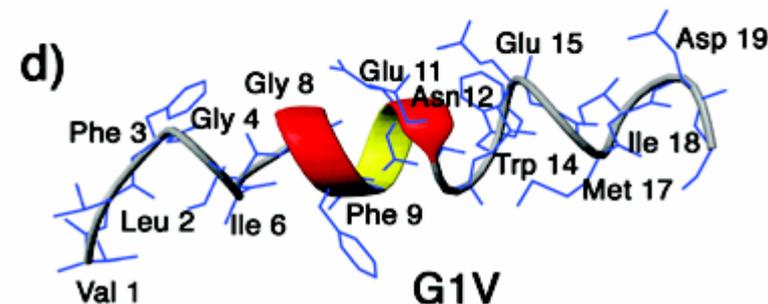
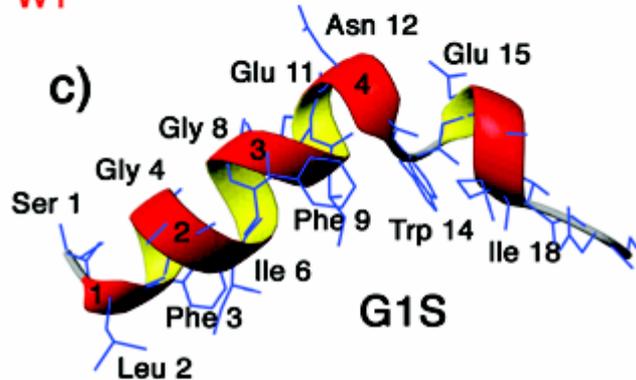
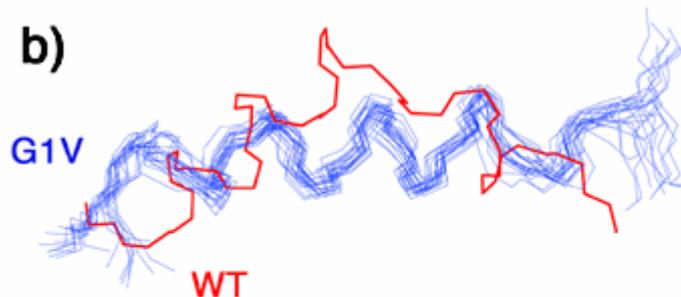
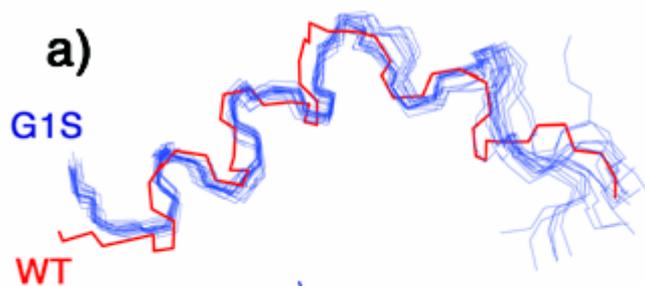
Immersion Depth of Spin-Labeled Side-Chains of Influenza HA Fusion Peptide in Lipid Bilayers Determined by Power-Saturation EPR Spectroscopy



Docked Fusion Peptide Structures in Outer Leaflet of Lipid Bilayer

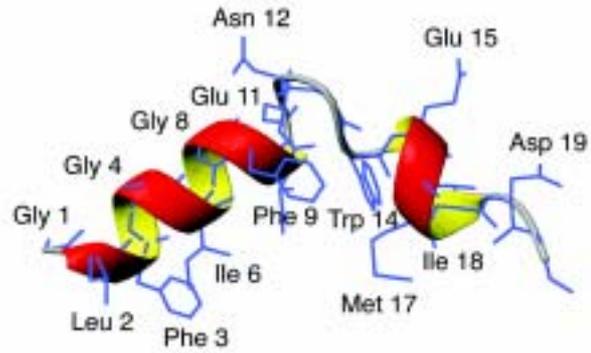


NMR Structures of Wild-Type and Mutant Fusion Peptides in DPC Micelles

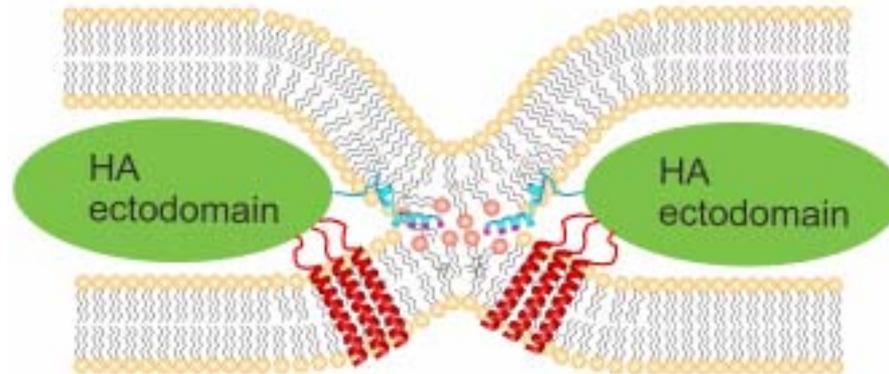


Lipid Mixer Model of Fusion Intermediate

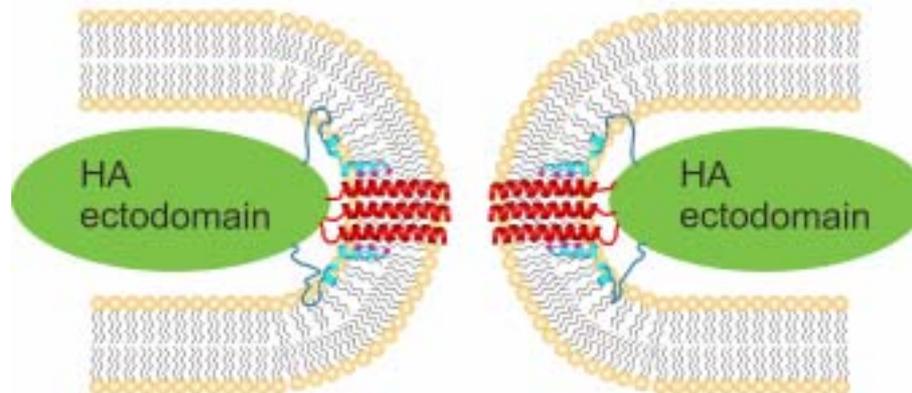
A



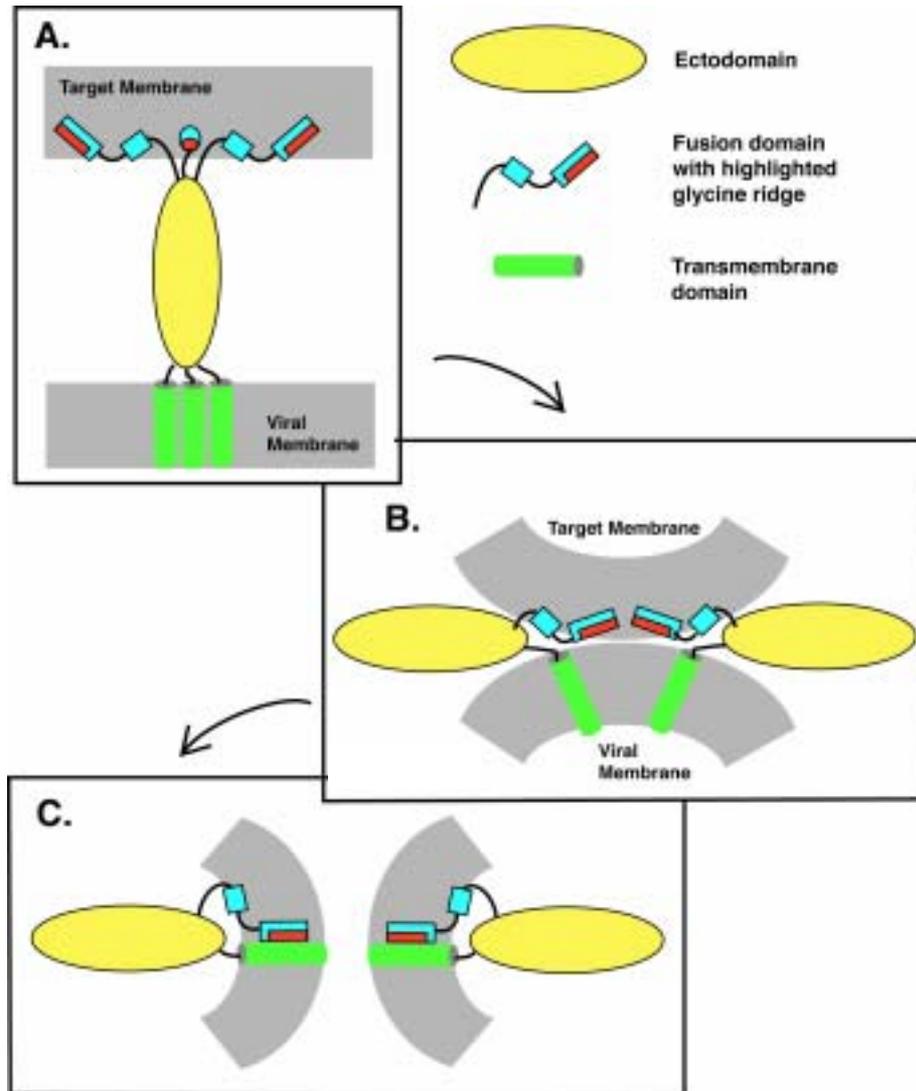
B



C



"Spring-Loaded Boomerang" Mechanism of Influenza Hemagglutinin-Mediated Membrane Fusion





Thanks to:

Yinling Li

Alex Lai

Xing Han



Cameron Gray

Suren Tatulian

Peter Hinterdorfer



Collaborators

John Bushweller, University of Virginia

David Cafiso, University of Virginia

Judith White, University of Virginia



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